INHIBITION OF GLUCAGON-STIMULATED ADENYL CYCLASE BY INSULIN

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1. Introduction

The studies of Jefferson et al. [1] and of Menahan and Wieland [2] provided evidence for a regulatory role of 3′, 5′-AMP in the antagonism of glucagon and insulin on the isolated perfused liver. From the fact that insulin counteracted the effect of glucagon on gluconeogenesis, urea production and ketogenesis, but failed to antagonize the stimulation of these parameters by N^2, O^6-dibutyryl-adenosine 3′, 5′-phosphate, an interaction of these two hormones at a step at or prior to the formation of the cyclic nucleotide was proposed [2]. In adipose tissue homogenates, Jungas observed an inhibition of net Ado-3′, 5′-P formation after previous treatment of the tissue with insulin [3] and from the work of Butcher et al. [4], and Hepp et al. [5] an effect of insulin on the adenyl cyclase system of isolated fat cells could be inferred. However, several groups who where studying adenyl cyclase in different preparations from adipose tissue, did not find a significant effect of insulin *in vitro* [6–9]. On the other hand, a protein fraction prepared from mammalian serum and termed non-suppressible insulin-like activity (NSILA) [10, 12] was shown to inhibit the formation of 3′, 5′-AMP in adipocytes [9]. In the course of studies on glucagon action upon adenyl cyclase activity in mouse liver [11] an antagonistic action of insulin and NSILA was observed. In the present communication these effects are described and a possible mechanism of action in the complex receptor–enzyme system is proposed.

2. Materials and methods

Particulate homogenate fractions were prepared from fresh livers of male Swiss albino mice (35–40 g) which were fed a normal laboratory chow *ad lib*. The method as well as the test for adenyl cyclase activity have been described in detail [11]. Crystalline bovine insulin (27 U/mg, lot No. 21/024) was obtained from Farbwerke Hoechst, crystalline porcine insulin (25 U/mg, lot No. S 13868, 10 times recrystallized) from the NOVO Company. Dilutions were made in aqueous solutions of 3 mg/ml crystalline bovine albumin (Behringwerke), resulting in a final concentration of 300 μg/ml in the test. Control samples received albumin solutions alone. Synthetic glucagon was a gift of Dr. E. Wünsch, Max-Planck-Institut für Eiweiss- und Lederforschung, Munich. NSILA-S [12] preparations I, II and III were from the laboratory of Dr. R. Froesch, Zürich. Their specific biological activity per mg dry weight was equivalent to 0.1, 1 and 10 mU of crystalline insulin, respectively. Part of preparation I was inactivated by reduction and aminoethylation which resulted in the complete loss of its biological activity. Control samples contained an equal amount of protein as crystalline albumin. All hormones were added to the test mixture before starting the reaction with the addition of the liver preparation.

3. Results

3.1. *Effects of crystalline insulin*

Table 1 shows the effect of 100 μU/ml of crystalline bovine insulin on adenyl cyclase activity which was submaximally [11] stimulated by synthetic glucagon (see also fig. 1). A 33% inhibition (of total activity) was observed which, on the basis of paired comparison, was highly significant. The effect could be reproduced with equal concentrations of porcine insulin. In the presence of calcium, which inhibits

Table 1

Effect of crystalline insulin of glucagon stinulated adenyl cyclase activity in mouse liver in presence and absence of calcium. Assay system according to [11]. ATP/Mg ratio 1:2.

Additions	No of expts.	Adenyl cyclase activity (nmoles X g protein^{-1} X min^{-1} ± S.E.M.)	Δ	p
None	6	6.3 ± 1.5	+ 22.6 ± 5.7	
Glucagon (0.5 μg/ml)	6	28.9 ± 6.1		
Glucagon (0.5 μg/ml) + insulin (100 μU/ml)	6	19.2 ± 6.2	- 9.7 ± 1.7	<0.001
Ca^{2+} (1 mM)	5	3.1 ± 1.1		
Ca^{2+} (1 mM) + glucagon (0.5 μg/ml)	5	13.6 ± 2.8	+ 10.5 ± 2.1	
Ca^{2+} (1 mM) + glucagon (0.5 μg/ml) + insulin (100 μU/ml)	5	5.9 ± 1.5	- 7.7 ± 2.1	<0.01

both the basal and the glucagon effect, the inhibition was relatively stronger (56%). Fig. 1 shows a dose response curve of glucagon in presence and absence of bovine insulin. Although an inhibition could be also observed when maximal doses of glucagon were present, the curves shown can not exclude a competitive mechanism. It should be noted that the present assay system contained concentrations of ^{32}P-ATP which were far below saturation, and that no methylxanthines were present [11].

3.2. *Effect of NSILA-S and RAE-NSILA-S*

NSILA-S, [10, 12], inhibited glucagon-stimulated adenyl cyclase over a concentration range which corresponds to its biological action (table 2). In contrast to its inhibition of the glucagon effect, no antagonism

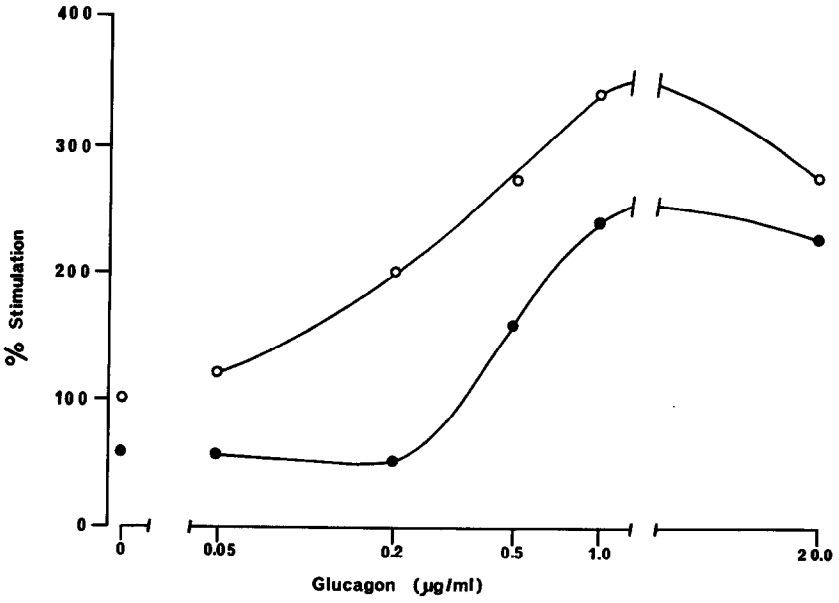

Fig. 1. Dose response curve of synthetic glucagon in presence (•) and absence (○) of 100 μU/ml of crystalline insulin. Data from two experiments are given as percent stimulation above basal values. ATP/Mg ratio 1:2.

Table 2

Effect of NSILA-S and biologically inactive RAE-NSILA-S on glucagon- or fluoride-stimulated adenyl cyclase activity in mouse liver. Biological activity is expressed as μU-equivalents, as standardized with crystalline insulin on rat adipose tissue *in vitro* [10, 12] Test system for adenyl cyclase according to [11]. ATP/Mg ratio was 1:10.

Additions	μU-equivalents/ml	Adenyl cyclase activity (nmoles × g protein^{-1} × min^{-1})
Experiment I		
Glucagon (5 μg/ml)		
+ albumin (200 μg/ml)	–	42.0
+ albumin (195 μg/ml) + NSILA-S III (5 μg/ml)	50	38.3
+ albumin (190 μg/ml) + NSILA-S III (10 μg/ml)	100	35.8
+ albumin (150 μg/ml) + NSILA-S III (50 μg/ml)	500	26.8
+ NSILA-S (200 μg/ml)	2000	18.1
Experiment II		
Albumin (500 μg/ml)	–	3.9
Albumin (500 μg/ml) + glucagon (5 μg/ml)	–	54.6
RAE-NSILA-S (500 μg/ml) + glucagon (5 μg/ml)	0	54.8
NSILA-S I (500 μg/ml) + glucagon (5 μg/ml)	50	30.0
Albumin (500 μg/ml) + NaF (10 mM)	–	70.6
NSILA-S II (500 μg/ml) + NaF (10 mM)	500	84.3

to fluoride stimulation could be observed (table 2). Furthermore, when a different preparation (NSILA-S I) was inactivated by reduction and aminoethylation, it had also lost its effect on the adenyl cyclase system. A non-specific effect of these still impure fractions seems thus unlikely.

4. Discussion

In a recent publication, Ray and coworkers have described an effect of very high concentrations of insulin on basal and glucagon-stimulated adenyl cyclase activity of isolated plasma membranes of rat liver [13]. Since the properties of their adenyl cyclase preparation differ in several respects from those seen with the present mouse liver fraction [11], entirely different enzyme systems may have been under study. In the present communication, physiological concentrations of insulin were shown to be active.

In view of the well known sensitivity of the mouse to insulin *in vivo* it seemed advantageous to investigate the effect of this hormone on an adenyl cyclase preparation from mouse liver. Indeed, when similar rat liver preparations were studied in preliminary experiments, the effects were smaller and more variable. Other factors, such as the absence of the methylxanthines, the presence of albumin, and the use of submaximal concentrations of synthetic glucagon in the assay, may have been helpful. Of interest is the finding of a relative increase of the insulin effect in the presence of Ca^{2+}. In view of the observation that insulin enhanced the binding of Ca^{2+} to artificial membranes [14] it seems attractive to speculate that the hormone may antagonize glucagon action in such a way. Since even highly purified insulin is not a homogeneous substance, it is not absolutely certain that the insulin molecule itself has caused the effect. It should be noted, however, that several insulin preparations of different origin were active in the physiological concentration range. Since NSILA shares many metabolic effects with crystalline insulin, it represents an interesting model substance. Since preliminary experiments showed that its interaction with glucagon was non-competitive, maximal glucagon concentrations were used. The fact that NSILA inhibits the glucagon but not the fluoride stimulated cyclase system, represents an additional difference in the characteristics of glucagon and fluoride stimulation [11]. It suggests that insulin activity may interact with glucagon directly at its receptor site or with the transmission of the hormone signal to the catalytic

(fluoride stimulated) unit of the adenyl cyclase system.

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